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Antibody Heterogeneity and Serological Reactions

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INTRODUCTION

The antibody activities of serum are attributable to a family of globulins which can be distinguished by their chemical, physical, and antigenic properties. The extreme heterogeneity of these immunoglobulins (35) with respect to electrophoretic mobility, molecular size, and structure has been emphasized in a succession of reviews of which several have appeared within the year (39, 44, 81). A standard system of classification and nomenclature (27) has been recommended to avoid the confusion which would inevitably result from a diverse terminology. As the criteria for recognizing the different classes of immunoglobulins have become better defined, it has been increasingly apparent that immunological reactivity is often dependent upon the kind of antibody present. Although an antibody of one class can participate in several immunological reactions in compliance with Zinsser's unitarian theory (157), one kind of antibody may be much less efficient than another or may fail completely to bring about a given reaction. Our present purpose is to summarize recent information regarding the capacity of different immunoglobulins to elicit various serological reactions. Although it is impossible to separate completely the serological activity of antibodies from other phases of antibody function, no attempt will be made to review the many biological problems concerned with antibody formation and distribution. The cellular origin of immunoglobulins (80, 152) and immunological memory (142) are important related subjects not included in this discussion.

The three major classes of immunoglobulins known to have antibody activity are γG (IgG), 7*S*, usually mercaptoethanol-resistant; γM (IgM), 19*S*, mercaptoethanol-sensitive; and γA (IgA) which may occur as 7 to 17*S*, depending on the degree of polymerization (41). Two other immunoglobulins have been identified: γD, which accounts for less than 1% of normal human serum immunoglobulin (115*a*), has not yet been shown to have serological activity; a globulin associated with human reagin has been designated γE (63). These five globulins have antigenically different heavy chains. In addition, subclasses and allotypes have been described (39, 41). The γG and γM are most readily isolated and identified, and have been most extensively studied in relation to immunological properties. The first portion of this review will, therefore, be concerned with a comparison of the activities of these two immunoglobulins. Since the serological activity of a given serum specimen may be influenced by the relative concentrations of these two classes of anti-

body, it also will be profitable to examine the circumstances which may affect their occurrence. Finally, the more limited information regarding γA will be summarized.

COMPARISON OF γG AND γM IMMUNOGLOBULINS

Sequential Appearance in Serum

According to the experience of most investigators, the first antibody to be detected in the serum after antigenic stimulation is γM. This initial antibody usually declines or disappears completely in several weeks to be replaced by γG which may persist for long periods of time. Such a sequential synthesis has been studied most extensively in the rabbit and has been reported in a wide variety of species (Table 1) in response to soluble and particulate antigens of diverse composition. The tabulation includes not only instances in which the sequence was observed after a single primary stimulus but also situations in which a secondary stimulus or hyperimmunization was required to elicit detectable amounts of γG. Although this pattern appears to be a general one, there are variations in the degree to which γM is replaced by γG, depending on the kind of antigen, the dosage, and the species. Of special interest is the fact that the early γM response in the turtle (52) and the goldfish (144) is unusually prolonged as compared to that observed in mammals. There are some reports in which no γG at all has been detected (10, 30, 79), but others have obtained a γG response to the same antigens. The relation of the antibody sequence to the cellular aspects of the immune response and some of its implications have been reviewed by Uhr (142).

There have been only a few opportunities to observe the sequence of antibody appearance in naturally occurring infections. Murray et al. (84, 85) have shown that the antibody in primary typhus is predominantly γM, whereas that in the recrudescent disease is γG. Serum from patients with leptospirosis taken from the 2nd to the 5th week after onset showed an increase in the proportion of γG as determined by agglutination of some serotypes but not of others during this time interval (93). In heifers (109) and human volunteers (103) experimentally infected with *Brucella*, the early agglutinin was γM; γG was not observed until about 2 weeks after infection.

More recent observations, however, suggest that the sequential synthesis of γM and γG may be more apparent than real. Freeman and Stavitsky (40), by means of radioimmunoelectrophoresis, detected the simultaneous appearance of γG and γM antibodies in rabbits after injection of human serum albumin and bovine γ-globulin.

TABLE 1. *Reports recording the early appearance of γM followed by the appearance of γG antibody activity*

Species	Antigen	Reference
Rabbit	Serum proteins	10, 11, 15, 53
	Hemocyanin	10, 11, 30
	Haptenes	9
	Diphtheria toxoid	11, 106
	Erythrocytes	11, 95, 129
	Salmonella H antigen	10, 11
	Salmonella O antigen	99, 147
	Leptospires	93
	Mycobacteria	29
	Poliovirus	132
	Bacteriophage	10, 11, 143
Mouse	*Salmonella*	140
	Actinophage	69
	Sheep red cells	3
	Ovalbumin	4
	Ferritin	4
	Influenza virus	18
Rat	Bacterial flagella	1
Guinea pig	Foot and mouth disease virus	24, 28, 50
	Bacteriophage	143
	Arbovirus	13
Cattle	*Brucella*	109, 110, 111
	Foot and mouth disease virus	24
Sheep	Diphtheria and tetanus toxoids	78
Man	*Salmonella* H antigen	79, 141
	Brucella	103
	Diphtheria toxoid	106
Chicken	Serum albumin	17, 105
	Bacteriophage	112, 144
Turtle	Hemocyanin and serum albumin	52
Frog	Bacteriophage	144
Goldfish	Bacteriophage	144

Using a method which measured the antigen-binding capacity of rabbit antibodies for human serum albumin, Osler et al. (89) found even greater quantities of γG than γM within the first week after antigen injection. Although the synthesis of γG and γM may begin at about the

same time, it is obvious that γM will appear to predominate in early sera if the methods used for measuring antibody activity, for example, agglutination, favor the detection of γM (40, 89).

Factors Influencing the Kind of Antibody Produced

Nature of the antigen. Protein antigens have usually been found to stimulate a preponderance of γG antibody after the early γM has decreased or disappeared (10, 53). The same has been true of viruses (18, 28, 50), bacteriophage (69, 112, 143), and simple haptenes (9). On the other hand, the lipopolysaccharide somatic antigens of enterobacteria stimulate γM antibodies predominantly in man and the rabbit (10, 79, 86, 97, 99, 147), whereas in the guinea pig the antibodies to *Escherichia coli* polysaccharide were almost entirely γG (22).

There is evidence that the physical state of the antigen may be a factor. Ada et al. (1) compared the response of rats to the monomer and polymer forms of flagellin and noted a greater tendency of the latter to stimulate γM. Lindqvist and Bauer (77) found that aggregated bovine serum albumin induced a consistently higher γM response in rabbits than did nonaggregated albumin. Rabbits immunized with thyroglobulin bound to acrylic resin particles showed a 20-fold increase in γM as compared to rabbits immunized with thyroglobulin alone (139). A marked γM response in rabbits was obtained by the injection of particulate deoxyribonucleic acid (DNA)-methylated bovine serum albumin complex (119). These observations strongly suggest that particle size may influence the kind of antibody produced.

Dosage of antigen. There are indications that the dosage of antigen or the intensity of antigenic stimulation can influence the proportion of γG and γM produced. It was first reported that bacterial lipopolysaccharide antigen elicited γM antibodies only (10, 79), but, when larger amounts of antigen were injected over longer periods of time, considerable amounts of γG were also demonstrable (97, 99, 107, 147). Only γM agglutinins for sheep erythrocytes were found after a single injection, whereas both γG and γM appeared after two injections in rabbits (11). Svehag and Mandel (132) observed that a relatively small dose of poliovirus in rabbits elicited only γM, but, when a much larger dose was given, the initial γM was followed by γG. It must be recognized, of course, that the greater the total quantity of antibody, the greater the chance of being able to detect a given proportion of a minor component. The relative efficiency of a serological procedure in detecting different classes of antibody must also be considered (89, 99).

Other factors. A variety of other factors may also influence the kind of antibody produced. Treatment of mice with thorotrast prolonged the γM response to *Salmonella typhimurium*, as measured by opsonic activity, and increased the γM response to sheep cells, while completely inhibiting the production of γG (65). Endotoxin injection and X irradiation prolonged the γM response to bacteriophage (143). That different species may respond differently to the same antigen was shown by Dixon et al. (30), in that rabbits produced both γM and γG after intravenous injection of hemocyanin, whereas rats produced γM only. There was a γG response in rats, however, when the antigen was mixed with Freund's adjuvant.

It is apparent from these examples that both γG and γM antibodies can result, under the proper circumstances, from the injection of any antigenic material so far studied, although one or the other may predominate in a particular situation. There are several naturally occurring antibodies, however, which thus far have been found only as γM globulins. The natural antibodies of the mouse for sheep erythrocytes (3, 151), and the normal rabbit antibody for the poliovirus (131) are in this category, although γG with the same specificity can be produced by artificial immunization. At first, the only natural antibodies of man detected by reactions with gram-negative bacteria were γM (34, 82), but, by indirect fluorescent-antibody staining, a major portion of natural human antibody to *Escherichia coli* and *Neisseria gonorrhoeae* was found to be γG (26*a*, 26*b*). Differences in the sensitivities of the tests used probably accounts for the earlier failures to detect natural γG (26*b*). The cold agglutinins which occur in patients with hemolytic anemia (42) and with pneumonia due to *Mycoplasma* (71) have been identified as γM with the exception of one instance in a patient who had cold agglutinins of the γA class (6). Although the sheep cell antibody developing in infectious mononucleosis, which is completely absorbed by boiled beef cells, is γM (71, 76, 91, 94), antibody produced in rabbits by the injection of boiled beef cells was found in both γM and γG fractions (24*a*). The rheumatoid factors which are usually considered to be antibodies to normal γ-globulin have the same physicochemical and antigenic properties as other γM antibodies (73). There is increasing evidence, however, that rheumatoid factor activity may also be present in γG and γA globulins (55).

Heat Inactivation

It has been known for many years that antibodies differ with respect to resistance to inacti-

vation by heat, and that heat resistance tends to increase with time after immunization (74). Kidd and Friedewald (68) described a naturally occurring antibody in rabbit serum which fixed complement with extracts of rabbit tissues, and found that its activity was destroyed by heating at 65 C for 30 min; specifically induced antibodies were not affected by this temperature. They discussed the significance of this antibody in relation to "nonspecific" complement-fixation reactions observed by numerous other investigators, and noted that such reactions could frequently be avoided by heating the serum to a degree which would not affect specific antibodies. Natural sheep hemolysin and Wassermann reagin found in normal rabbit serum showed a heat lability comparable to the antibodies for rabbit tissues (68). Studying autoantibodies produced in rabbits by the injection of rat tissue, Asherson and Drumonde (7, 8) found that macroglobulin antibody was destroyed by heating at 65 C for 30 min, whereas the low molecular weight antibody was stable at this temperature.

The recognition of differences in heat inactivation of *Brucella* agglutinins has received practical application in tests to distinguish nonspecifically occurring agglutinins from agglutinins resulting from infection (149). Two types of nonspecific agglutinins have been detected, both of which are more susceptible to heat inactivation than are the specific agglutinins (149). The nonspecific agglutinins resemble the early postvaccinal or postinfection agglutinins in heat lability and in sedimenting more rapidly on ultracentrifugation than the low molecular weight agglutinins which appear later (109–111).

As long ago as 1904, it was observed that agglutinins for bacterial flagella were more stable on heating than were agglutinins for somatic antigens (19). This observation was repeatedly confirmed over a period of 50 years without adequate explanation (98). When it was reported that somatic agglutinins tended to be γM whereas antiflagellar antibodies were predominantly γG (10), Pike and Schulze prepared antisera to several antigens and compared the heat stability of γG and γM antibodies to each of these antigens (98). The γG antibodies to the typhoid somatic antigen withstood a temperature of 70 C for 30 min, whereas the γM antibody activity was partially destroyed at 70 C and completely inactivated at 75 C. The same relative susceptibilities to heat were shown for γG and γM antibodies to sheep cells, leptospires, bovine serum albumin, and the flagellar antigens of the typhoid bacillus. It became apparent that the observed difference in heat stability of somatic and flagellar agglutinins was merely a reflection of the relative heat stability of the predominant type of antibody globulin involved.

This difference in heat lability of γG and γM seems to be a consistent finding, since it has been shown for tetanus and diphtheria antitoxins produced in sheep (78) and for rabbit antibodies for the poliovirus (131). Locke and Segre (78) found that inactivation at 65 C for 1 hr provided a more reliable measure of macroglobulin antibody than did treatment with mercaptoethanol. This difference in heat resistance is compatible with the well-known susceptibility of normal antibody to heat (68, 124) and the finding that most normal antibodies are γM (124, 131).

Stability of Union with Antigen

The firmness with which antibody binds to antigen is a property often referred to as avidity. Less avid antibody dissociates from its union with antigen more readily than does antibody of high avidity. The literature pertaining to this subject is sometimes difficult to interpret because of the varied criteria used for measuring avidity. The effect which firmness of attachment may have on the end result of an antigen-antibody reaction depends on the kind of reaction involved. When lysis of erythrocytes or bacteria is the end result, an antibody which can more readily dissociate from antigen may produce serial injuries to cells although only a limited amount of antibody is present. When neutralization of a toxin or a virus is the end result, dissociation would liberate toxin or infectious virus, and antibody activity would therefore be less effective. The well-known fact that antitoxins produced in the early stages of immunization tend to have irregular neutralizing power and are easily dissociated from toxin on dilution (102) would suggest that γM may be in general less avid than γG. The greater bactericidal effect of γM (107), as well as its inability to neutralize toxins and enzymes (106), would also imply low avidity.

In some instances in which the avidity of γG and γM antibody has been compared, the data seem to support the suggestion that γM is less avid. Robbins et al. (107) found that γM dissociated from *S. typhimurium* more readily in acid solution than did γG. Furthermore, absorption preferentially removed γG (58). In virus-antibody mixtures, Svehag (131) showed that infectious virus was released more readily from γM in acid solution than from γG, indicating a greater avidity of γG.

Other experiments have led to different conclusions. One method used to measure the stability of the union between antibody and erythrocytes

has been to determine the degree of cell-to-cell transfer. Taliaferro (134) and Goodman and Masaitis (47) found that rabbit γG anti-sheep hemolysin was transferred to a greater extent than γM, indicating a greater avidity for γM. Greenbury et al. (51) also concluded that γM is more avid than γG, since a given number of human A cells fixed a considerably greater proportion of the γM antibody than of the γG. By measuring the degree of dissociation of bacteriophage from guinea pig antibodies, Finkelstein and Uhr (38) found that γM obtained 1 week after immunization had a higher avidity than γG obtained 1 week later.

Attempts to compare the avidity of γG and γM are complicated by the fact that the affinity of antibody increases with time after immunization (32, 53, 135), and the increase can occur in one molecular class of antibody (70, 128, 134). Studying changes in the quality of antibody during the course of immunization, Grey (53) found that, at the same stage of immunization, the dissociation rates of rabbit anti-bovine serum albumin γG and γM were similar. Even if there was a difference, insufficient γM was present to account for the observed change in binding strength with time. The avidity of both γG and γM guinea pig antibodies for bacteriophage increased with time after immunization (38). In the light of these observations, any comparison of the avidity of different classes of antibody should take into account the time factor as well as other considerations discussed by Finkelstein and Uhr (38).

Agglutination

It is apparent from many observations that both γG and γM antibodies are capable of causing agglutination, but to different degrees. Greenbury et al. (51) compared rabbit ^{131}I-labeled γG and γM antibodies for human erythrocytes and found that over 100 times as much γG as γM was required to give 50% agglutination (Table 2). On a molecular basis, they calculated that 19,000 molecules of γG per cell were required for 50% agglutination, whereas only 25 molecules of γM per cell were required. Other reports (57, 64, 77, 99, 107, 133) giving quantitative data show considerable variation (Table 2), but most agree that smaller amounts of γM than γG are required for agglutination. One notable exception is the report of Heremans et al. (57) in which human γG and γM displayed approximately the same agglutinating activity for *Brucella*. In a passive agglutination test, the same authors found that considerably more γM than γG antidiphtheria toxin was required for agglutination. Other reports in which passive agglutination was employed to demonstrate human antibodies to penicillin (146), and to demonstrate rabbit (53) and chicken (105) antibodies to bovine serum albumin, indicate higher agglutinating activity for γM without giving quantitative comparisons. Although the diversity of antigens, reaction end points, and methods of assay used in these studies would be expected to result in variations in the quantitative data, it is unlikely that this could account for all the differences observed. An additional important variable is evident from the work of Osler et al. (89), who found that γG rabbit anti-human serum albumin obtained from early bleedings after a single injection possessed feeble if any hemagglutinating activity, in contrast to γG antibody from hyperimmune sera which showed higher specific agglutinating activity than γM from the same sera. Furthermore, there was considerable variation in the agglutinating properties of the immunoglobulins of different animals.

It is apparent from most of the work cited that agglutination tends to favor the detection of γM antibody, particularly if early sera are examined. In comparing the agglutinating efficiency of the two immunoglobulins, it is important to select an appropriate basis for comparison. To note that one serum fraction has a higher agglutinin titer than another merely describes the distribution of antibody activity in the sample of serum. Comparing agglutinating activity with some other serological manifestation which itself may be variable can give a distorted impression. The absolute amount of antibody expressed as the weight of antibody nitrogen or the molecular concentration would, therefore, seem to be a more appropriate basis for comparison.

Precipitation

Studying rabbit antibodies to bovine serum albumin, Benedict et al. (15) obtained evidence that hemagglutination detected antibodies which could not be demonstrated by precipitation. Several investigators have reported little or no precipitation with γM antibodies for serum albumin (53, 105, 123), although these antibodies were readily detected by passive hemagglutination. Both γM and γG, however, gave precipitin lines in gels with *Salmonella* endotoxin (107, 147), with soluble rickettsial antigen (84), and with coccidioidin (120). Mulholland et al. (83) reported quantitative precipitation results with *S. enteritidis* endotoxin and rabbit sera which contained predominantly γM antibody. It is well known that rheumatoid factor, a γM globulin, reacts with normal γ-globulin to form a precipi-

TABLE 2. *Agglutinating activity of γG and γM antibody*

Species	Antigen	Agglutinating activity per ml		Reference
		γG	γM	
Rabbit	Human erythrocytes	0.45–0.59 μg of protein[a]	0.004–0.0045 μg of protein[a]	Greenbury et al. (51)
Rabbit	Human erythrocytes	0.05 μg of N[b]	0.004 μg of N[b]	Tada and Ishizaka (133)
Human	Human erythrocytes	0.01 μg of N[b]	0.0004–0.0008 μg of N[b]	Ishizaka et al. (64)
Rabbit	*Salmonella typhimurium*	25–50 μg of protein[b]	1–5 μg of protein[b]	Robbins et al. (107)
Rabbit	*S. typhosa*	1.44 μg of protein[a]	0.13 μg of protein[a]	Pike et al. (99)
Human	*Brucella*	10.9 μg of protein[b,c]	9.4 μg of protein[b,c]	Heremans et al. (57)
Rabbit	Human serum albumin	0.29 μg of protein[a]	0.15 μg of protein[a]	Lindqvist and Bauer (77)
Human	Diphtheria toxin	1.6 μg of protein[b,c]	7.5 μg of protein[b,c]	Heremans et al. (57)

[a] Amount required for 50% agglutination.
[b] Minimal amount required for agglutination.
[c] Based on total purified immunoglobulin.

tate (33, 56). Tada and Ishizaka (133) obtained precipitation with both γG and γM antibodies to human blood group A substance, but the flocculation time for γM was much slower, and inhibition of precipitation by excess antigen was less apparent with γM than with γG. The slow flocculation time of γM is consistent with the observation of Grey (53) that γM had a slower association rate with antigen than γG, possibly due to differences in molecular charge and size. The relative insolubility of the γM precipitate in antigen excess has also been observed with rabbit antibody to typhoid endotoxin (99) and with rabbit antibody to bovine serum albumin (77). In all three instances, γG precipitin curves showed typical inhibition of precipitation in the region of antigen excess.

In studying the precipitating and agglutinating capacities of γG and γM antibodies to typhoid somatic antigen, it was found that γG was a much more effective precipitin than γM when agglutinin titer was used as the basis for comparison (99). When precipitin curves were determined for the two classes of antibody with varying amounts of endotoxin, the ratio of antigen to antibody at equivalence was roughly the same in both curves. This suggests that the ability of γG and γM to precipitate this antigen was of the same order of magnitude when compared on a weight basis.

There is no clear explanation for the contrasting agglutinating and precipitating capacities of γG and γM antibodies. The probability that the γM molecule has five (77) or more (60, 86*a*) combining sites may account in part for its marked agglutinating ability, but one would expect an analogous advantage to show up in precipitation.

Complement Fixation

Prior to 1960, it had often been observed that the ability of various sera to fix complement was variable with respect to other serological properties. For example, in virus infections complement-fixing activity of serum may appear later than other demonstrable antibodies (136). In studying the antibody response of guinea pigs to foot-and-mouth disease virus vaccine, Graves et al. (50) found that neutralizing antibody was demonstrable in the serum within the first week after inoculation, whereas complement fixation was not obtained until the 16th day. This delay in the appearance of complement-fixing activity was explained by showing that the early antibody was predominantly γM. It was not until over 50% of the neutralizing activity was found in the γG fraction that complement-fixing activity could be detected. This provided a clear illustration of the superior complement-fixing capacity of γG antibody reacting with a viral antigen. A similar situation has been described in guinea pigs inoculated with several arboviruses (13); the γM fractions of serum did not fix complement, and this was invariably associated with γG antibody. Thus, a probable explanation is provided for the observed delay in the appearance of complement-fixing antibodies in a number of virus diseases.

The relation of complement fixation to the classes of antibodies to a bacterial antigen has been shown in brucellosis. Heremans et al. (57) found that both γG and γM antibody obtained from the serum of a patient with brucellosis fixed complement, but the γG was markedly more effective than the γM. In comparing the agglutinating and complement-fixing activity of

bovine sera and milk against *Brucella*, Anderson et al. (5) observed that failure to fix complement was correlated with the presence of mercaptoethanol-susceptible agglutinins. Sera and milk containing mercaptoethanol-resistant agglutinins fixed complement well. After inoculation of calves with live *B. abortus*, the complement-fixing titer of the serum paralleled the titer of mercaptoethanol-stable agglutinin. These observations may be significant in relation to the diagnosis of chronic brucellosis in man, since Reddin et al. (103) have reported that the sera of patients with bacteriologically proven chronic brucellosis consistently contained mercaptoethanol-resistant agglutinins; in contrast, the sera of patients in whom the only evidence of chronic infection was a low agglutinin titer contained mercaptoethanol-susceptible agglutinins. These authors suggest a re-evaluation of the complement-fixation test for significance in the diagnosis of brucellosis.

A very striking example of the difference in complement-fixing properties of γG and γM was provided by the studies of Murray et al. (84, 85), comparing the serological reactions in primary epidemic typhus with those in recrudescent typhus (Brill's disease). The complement-fixation reaction in primary typhus was usually negative unless a relatively large amount of antigen was used (a characteristic designated as high antigen requirement), and inactivation of sera at 60 C instead of 56 C caused a marked reduction in titer. In contrast, complement fixation was readily obtained with sera from patients with recrudescent typhus by use of 1 unit of antigen, and the reactivity of the sera was not altered by inactivation at the higher temperature. Furthermore, the Weil-Felix titers, which depend upon the agglutination of *Proteus*, tended to be high in primary and low in recrudescent typhus. These differences in serological patterns were explained by showing that the predominant antibody in primary typhus is γM, whereas that of recrudescent typhus is γG. Thus, in addition to the relative heat lability and marked agglutinating properties of γM, the weaker complement-fixing activity of this class of antibody was shown in relation to the natural history of an infectious disease in man. It is of interest to recall that Zinsser, a strong proponent of the unitarian theory, expended great effort to show that typhus fever and Brill's disease, now known to be recrudescent typhus (84), were due to the same infectious agent (158).

The fixation of complement in relation to the type of antibody has been further elucidated by the identification of two varieties of low molecular weight antibodies in guinea pigs which are designated 7*S* γ1 and 7*S* γ2 according to their electrophoretic mobility (14). The 7*S* γ2 fixes complement (21) but does not attach to the skin to give passive cutaneous anaphylaxis (PCA; 90), whereas the 7*S* γ1 fails to fix complement but does give PCA. That this situation is not peculiar to the guinea pig is shown by the finding of similar antibodies in mouse antihemocyanin serum (36) and in horse antihapten serum (70). The 7*S* γ1 component of horse serum did not fix complement, whereas the 7*S* γ2 component did.

Resistance to inactivation by mercaptoethanol (54) has been widely used as a simple means of distinguishing γG from γM, although partial inactivation of chicken γG has been observed (16). It now appears, however, that, although some of the serological activity of γG may not be affected by mercaptoethanol, this compound destroys most of its complement-fixing ability (150) as well as other complement-requiring functions (127). Schur and Christian (122) suggest that complement fixation is associated with those double bonds which are more labile to mercaptan reduction. The complement-fixing titer of human γG for *Mycoplasma pneumoniae*, however, was not reduced by treatment with mercaptoethanol (121).

Lysis

Since the lysis of erythrocytes by antibody is dependent upon the presence of complement, it might be predicted that the type of antibody more effective in fixing complement would also have the stronger lytic action. Apparently, the reverse is true. In 1957, Stelos and Talmage (129) reported that γM rabbit antibodies for sheep cells were from 50 to 100 times more efficient in causing hemolysis than were γG antibodies when compared on the basis of combining power. On a molecular basis, it was estimated by Wigzell et al. (152) that mouse γM was 100 to 1,000 times more efficient than γG. A convincing demonstration of the greater lytic effectiveness of γM has been provided by Humphrey and Dourmashkin (60). Electron microscopic examination of sheep cells lysed by antibody and complement revealed the sites of damage as holes in the cell membrane. It was calculated that two to three molecules of γM antibody were sufficient to make one hole, whereas about 1,000 times as many molecules of γG were required for this effect. Observations by Goodman (46) and by Goodman and Masaitis (48) revealed a lack of correlation between the amount of complement fixed and the hemolytic activity of serum. Since the antibody with the greater molecular weight fixed less complement per hemolytic unit, it appeared that this antibody utilized complement more efficiently in the hemo-

lytic process than did the antibody of lower molecular weight.

The difference in the hemolytic efficiency of γG and γM has been apparent in the application of the plaque technique for detecting antibody for erythrocytes produced by single cells (67) and in the adaptation of this technique for the detection of cells producing antibodies to bacterial antigens (75). The parallel appearance of plaque-forming cells and γM serum antibodies indicated that only γM-producing cells were being detected by this method (75, 117, 152). When anti-γG serum is added to the system, however, plaques appear in the region of γG-producing cells (31, 130).

Bactericidal Activity

The killing of gram-negative bacteria by antibody and complement not only provides a highly sensitive method for detecting antibody, but is also presumably an important defensive mechanism. The bactericidal activity of normal human serum for aerobic enteric bacteria was found to reside almost entirely in the γM fraction (82). Landy et al. (75) found over 99% of the bactericidal antibody for *S. enteritidis* in rabbit serum after a single injection of endotoxin to be γM. Just recently, the bactericidal effect of normal human serum on some of the anaerobic, gram-negative bacteria indigenous to the oral cavity and intestinal tract also has been identified with the γM globulin (34). Spitznagel (127), however, reported that the normal guinea pig antibody responsible for the release of ^{32}P from labeled *Escherichia coli* in the presence of complement is γG. If this is the same antibody as that which is bactericidal, normal antibody in man and guinea pig must belong to different classes. Although the normal bactericidal antibody has usually been identified as γM, γG antibody against gram-negative organisms can be produced by hyperimmunization (97, 147), and this also has bactericidal activity. It appears to be less effective, however, than γM in relation to agglutinating capacity (147). Quantitative comparisons made by Robbins et al. (107) showed that on a weight basis 18 times as much γG as γM was required to kill 50% of a standard sample of *S. typhimurium*. The destructive effect of γM antibody for both erythrocytes and bacteria thus seems to be relatively more efficient than that of γG.

Neutralization

Antibodies have the capacity to neutralize toxins, enzymes, and viruses without participation of complement. Neutralizing activity has been found in both γG and γM antibodies for foot-and-mouth disease virus (23, 24, 28), poliovirus (12, 131, 132), influenza virus (12), several arboviruses (13), and bacteriophage (143). As a rule, virus-neutralizing power has been first detected in the γM, but, in sera collected later after immunization, γG becomes the predominant neutralizing antibody. A possible exception was noted in bovine serum in which the major portion of the neutralizing antibody for the virus of bovine diarrhea was γM (37). These studies show the distribution of neutralizing activity in various serum fractions, but offer no comparison of the relative efficiency of γM and γG on the basis of equivalent concentrations of antibodies. Svehag, however, did show that normal γM antibody for poliovirus formed weaker complexes with virus than did immune γG antibody (131).

Over the past 20 years, much has been written concerning the heterogeneity of antitoxic antibodies, indicating variations in ability to precipitate toxin and to neutralize it (102). Much of this information is difficult to correlate with the classes of immunoglobulins as currently described. Of special interest is the recent report of Robbins (106), showing that γM diphtheria antitoxin produced in rabbits did not inactivate toxin, although it did combine with toxin and caused strong indirect hemagglutination. The γG was capable of producing both agglutination and inactivation of the toxin. Likewise, γM combined with lysozyme but did not neutralize its enzymatic activity, whereas γG did neutralize this enzyme. The reported ability of γM antidiphtheria toxoid to neutralize at 5 C but not at 23 C (11) should be further explored.

Opsonization

Very few observations on the relative abilities of γG and γM to prepare antigenic particles for phagocytosis have been reported. The opsonic effect of pig serum for *S. typhimurium* was associated with a macroglobulin (66). Michael and Rosen (82) found the γM fraction of normal human serum more effective than γG in clearing *S. typhosa* from the blood of mice, but this merely indicated the location of the antibody activity and did not constitute a comparison of specific activity. Turner et al. (140) found that both γG and γM promoted phagocytosis of mouse typhoid bacilli, as determined indirectly by the clearance of organisms from the peritoneal cavity after injection. Since this was the only means of antibody assay used, no comparison of the relative efficiency of the antibodies could be made. The γG and γM antibodies in chick sera for goat erythrocytes showed parallel opsonizing and agglutinating titers (126). This suggests that γM

is the more effective opsonin, since it is definitely the more effective agglutinin.

Using removal of *S. typhimurium* from the circulation of inoculated mice as a measure of opsonic activity, Robbins et al. (107) concluded that γM was 500 to 1,000 times as efficient as γG as an opsonin. On the contrary, Smith et al. (125) found that γG rabbit antibodies to *P. mirabilis* were more effective than γM in promoting the ingestion of these bacilli in a leukocyte-bacterial system without complement. These apparently contradictory observations were not based on comparable experiments, however, since ingestion of bacteria by phagocytes is only one of several factors determining blood clearance.

Rowley et al. (118) showed that immunity to mouse typhoid could be transferred passively by means of phagocytic cells from immune mice. In this instance, at least, it was claimed that so-called cellular immunity was dependent on γM antibody adsorbed to cells, and that this antibody could be eluted from the cells with urea. The possibility that this phenomenon might influence the outcome of experiments involving phagocytosis should be considered.

Hypersensitivity

The varied reactons which are known to be manifestations of hypersensitivity are so diverse that one would hardly expect the same kind of antibody to be involved with all types. Generalities in this area are hazardous because of species differences in the antibodies involved. A discussion of the many aspects of this subject is available (26) and this subject is beyond the scope of this review. Some recent observations on the participation of molecular types of antibody in allergic phenomena, however, seem to be pertinent.

Two varieties of 7*S* antibody have been recognized in the guinea pig (14, 148). The 7*S* γ2, which appears to be analogous to the γG of man, gives only weak (148) PCA or none at all (90). The 7*S* γ1 antibody mediates both PCA and systemic anaphylaxis (90, 148). The similarities and differences between these γ1 antibodies and the anaphylactic antibodies of man, dog, rabbit, mouse, and rat, all of which migrate electrophoretically faster than γ2 globulins, have been extensively reviewed by Bloch (20*a*). PCA associated antibody of the rat sediments between the 7*S* and 19*S* components, is destroyed by heating at 56 C for 30 min, and is present in low concentration (20).

Osler et al. (89) found rabbit γM to be ineffectual in PCA, as did Lindqvist and Bauer (77). Human γM, according to Ovary et al. (91), did not give PCA in the guinea pig.

Both 7*S* γ1 and 7*S* γ2 of the guinea pig can passively sensitize to give the Arthus reaction (21). Furthermore, both γG and γM produced in the rabbit against human blood group A substance induced reversed Arthus reactions in guinea pigs, although the γM did not give PCA (133). More γM than γG was required to elicit a reaction.

Reactions Involving Rheumatoid Factors

One means of demonstrating factors in rheumatoid arthritis serum which react with γ-globulin is to show that such a serum will bring about the agglutination of erythrocytes sensitized with subagglutinating amounts of specific antibody (156). Rheumatoid factor reacts with the γ-globulin fixed to the cells as antibody. In studying the reaction of rheumatoid arthritis sera with sheep cells sensitized with various sheep cell agglutinins, two kinds of agglutinins were found which were not affected by rheumatoid factor. One was present in infectious mononucleosis serum (94), the other, in the serum of rabbits during the early stages of the immune response to the injection of erythrocytes (95). It then became apparent that only those antierythrocyte sera that contained appreciable amounts of γG antibody would sensitize cells for agglutination in rheumatoid arthritis serum (108). Infectious mononucleosis serum and early rabbit sera did not react with rheumatoid factor because the agglutinins were of γM type.

There are other ways in which the heterogeneity of immunoglobulin is involved in reactivity with rheumatoid factors. When γG is separated into three fragments by papain digestion, one fragment, the Fc, which contains only fragments of heavy chains, has no antibody activity, but does carry the antigenic determinants which enable it to combine with rheumatoid factor (49). The heterogeneity of these combining sites is reflected in the discovery that rheumatoid factors are actually a family of γM globulins, some of which have specificity for genetically determined antigenic groups present on the heavy chains of γG globulin (43). The differences that rheumatoid factors exhibit with respect to elutability from human γ-globulin suggested heterogeneity with respect to affinity (88). The possible relation of these differences to specificity for particular antigenic sites has not been determined. Although γG from a number of different species reacts with rheumatoid factor, reactivity is variable, and again indicates a multiplicity of rheumatoid factors (96). Other aspects of the multiple nature of rheumatoid factors have been reviewed (73).

γA IMMUNOGLOBULIN

The third principal immunoglobulin now designated γA is more difficult to identify than γG and γM (57, 145). It is usually inactivated by mercaptoethanol (64). As late as 1962, there was doubt as to the possible antibody activity of these globulins (35), although several investigators had reported antibody activity in serum fractions which contained γA and probably other globulins. Activity has now been demonstrated in γA from man (57), horse (70), bovine (153, 154), rabbit (87, 153), and guinea pig (115). Table 3 lists instances in which serological reactions have been ascribed to γA, as well as failures, to demonstrate such reactivity. Vaerman et al. (145) described a method for obtaining γA in a relatively pure form and demonstrated *Brucella* agglutinins in all three human immunoglobulins, including γA (57). Wilkinson (153) confirmed this observation and also showed *Brucella* agglutinin in analogous fractions of bovine and rabbit sera.

TABLE 3. *Immunological activity of γA immunoglobulin*

Activity	Reaction observed	No reaction observed
Agglutination	*Brucella* (57, 153), *Escherichia coli* (2), diphtheria toxin (57, 104), human erythrocytes (6, 64, 72, 100, 101, 138), streptococcal M protein (104), azo benzene sulfonate (24*b*)	
Precipitation	*Vibrio fetus* endotoxin (154), blood group A substance (64)	*p*-Azophenyl-β-lactoside (70), azo benzene sulfonate (24*b*)
Complement fixation		*Brucella* (57), human erythrocytes (6, 61*a*), *p*-azophenyl-β-lactoside (70)
Lysis		Human erythrocytes (64, 138), *E. coli* (2)
Neutralization	Poliovirus (12, 59), influenza virus (12), rhinovirus (114)	
Passive cutaneous anaphylaxis		Blood group A substance (64)

Evidence that γA globulin of human serum after typhoid vaccination combined with typhoid bacilli as antibody was presented by Rowe and Turner (116), but there was no direct evidence that any of the agglutinating activity of the serum was due to this globulin. The presence of γA antidiphtheria toxin in human serum was also shown by passive hemagglutination (57), but ability of γA to neutralize toxin was not studied. It has been suggested, however, that the component of immune horse serum which contains most of the antitoxic activity for diphtheria toxin is the equine equivalent of γA (57). Most of the precipitating activity of bovine serum for *Vibrio fetus* endotoxin was found in a fraction with the characteristics of γA (154). A portion of the insulin-binding antibodies (155) and some of the antithyroglobulin activity (45) of human serum was shown to be γA. Ishizaka et al. (64) observed precipitin reactions by the interfacial ring test with all three human immunoglobulins and blood group A substance. Reactions of γA, however, did not occur with all preparations, and they developed more slowly with γA than with γG and γM. Larger amounts of γA were required to bring about a reaction.

There are several reports indicating that some of the iso-hemagglutinins are γA (64, 72, 100, 101). Ishizaka et al. (64) compared the activities of different classes of human group A iso-hemagglutinins and found γA antibodies to be intermediate between γG and γM in their agglutinating activity, but this globulin failed to hemolyze erythrocytes and to give PCA. Klinman et al. (70) reported that equine antihapten γA failed to fix complement and to cause precipitation. It did, however, possess a high affinity for antigen and inhibited the precipitation of γA. Heremans et al. (57) had previously shown that human anti-*Brucella* γA did not fix complement. Cold hemagglutinins of the γA class in one individual did not fix complement or cause lysis (6). Rothman has recently described methods for isolating γA globulin from guinea pig serum (115), and showed that contact allergy to 2,4-dinitrochlorobenzene could be passively transferred by means of it. The possible relation of this antibody to the guinea pig 7*S* γ1 described earlier (14) has not been determined.

The evidence which tends to associate γA antibody with reagin, the heat-labile antibody found in the serum of patients with atopic allergy, has

been summarized by Chase (26). More recently, Ishizaka et al. (62, 63) have presented evidence that reagin can be distinguished from γA immunologically, although the physicochemical properties of reagins and γA are similar. They suggest that reaginic activity resides in a new immunoglobulin which they call γE. The possibility that reaginic activity may not be confined to this immunoglobulin has had recent support (79*a*, 91*a*).

One of the most intriguing characteristics of γA antibody is its tendency to be concentrated in certain secretions. Most of the neutralizing activity of human nasal secretions for poliovirus (12), influenza virus (12, 113), and rhinovirus (114) was due to γA antibody, even though the predominant neutralizing antibody in the serum was γG. The neutralizing antibody of human colostrum for the poliovirus (59) and the isohemagglutinin found in human saliva and colostrum (138) were likewise γA. There is evidence that reaginic activity of saliva (61) and nasal secretions (104) of pollen-sensitive individuals is present in γA globulin, but the identification of serum reagin with γE (62, 63) suggests that the reagin of secretions may also belong to this class. Antibodies for *Francisella tularensis* in human nasal secretions were associated with γA. The agglutinins for *E. coli* in human colostrum were found to be γA, but these antibodies were not lytic in the absence of lysozyme (2). There is very recent evidence that susceptibility of man to herpesvirus infection is, at least in part, due to a deficiency in the production of specific γA (137). Herpes infection, with its predilection for mucocutaneous tissue, is a situation where the neutralizing antibody of secretions might be expected to play an important protective role. Although rabbit serum contains a relatively low concentration of γA globulin, rabbit colostrum was found to contain a much higher concentration (25). That such a globulin may have antibody activity in the rabbit as well as in man has also been demonstrated (87, 153).

The origin of γA antibody in secretions is a question of some interest. In the rabbit, the goat, and the cow, there is evidence (92) that immune globulin is transferred from the plasma to the colostrum with considerable concentration being effected during the transfer. On the other hand, in man, Tomasi et al. (138) were unable to obtain transfer of ^{131}I-labeled γA from serum to saliva, and presented evidence for the local glandular synthesis of γA. They also showed that the γA of human colostrum and saliva differed from the major portion of serum γA in immunological specificity and in the degree of polymerization. These observations led them to conclude that external secretions derive antibodies from an immunological system separate from that of circulating antibody. This conclusion was supported by Adinolfi et al. (2), who found γA antibodies for *E. coli* in human colostrum in the absence of serum antibodies of this type. Whether the gland selectively concentrates γA or synthesizes it to the relative exclusion of γG and γM, an interesting problem is presented.

OUTLOOK

The recognition of different classes of immunoglobulins and the determination of their capacities to bring about the various immunological reactions have already led to a better understanding of several immunological phenomena. The necessity for considering the diverse properties of immunoglobulins in studying antibody production is being recognized more and more. Although the patterns of antibody formation and function in various species are generally similar, enough differences have been discovered to indicate that what is observed in one species is not necessarily true for others. It is inevitable that additional subtle differences in antibody properties will be correlated with immunological reactivities.

Although a change in the predominant class of antibody present in a serum can affect its serological activity, for example, complement fixation or agglutination, more information is needed regarding the possible changes in the activity of each class of antibody with time. It has been well established that the ability of antibody to form a stable union with antigen increases with time, but the extent to which this increased stability may be reflected in the observed result of the antigen-antibody union has not been fully determined. There are also indications that differences in the antibodies of individual animals may result in differences in observed activity (89).

The heterogeneity of the antibody globulins that are intermediate between γG and γM has not been completely defined. Information is lacking regarding the serological activity of γD and γE immunoglobulins, but the association of the latter with human reagin (63) suggests that it may also participate in other demonstrable reactions. With the aid of refined techniques, additional immunoglobulins present in low concentration may be identified. The relation of the intermediate antibodies of the guinea pig (8*a*, 14, 115, 148) to similar antibodies of other animals and man (20*a*) has yet to be clarified.

SUMMARY

In Table 4, an attempt has been made to indicate the comparative effectiveness of the principal

TABLE 4. *Summary of the activity of the immunoglobulins*[a]

Reaction	γG	γM	γA
Agglutination	Weak	Strong	+
Precipitation	Strong	Variable	±
Complement fixation	Strong	Weak	−
Lysis	Weak	Strong	−
Neutralization			
Viruses	+	+	+
Toxins and enzymes	+	−	?
Passive cutaneous anaphylaxis	−[b]	−	−
Arthus reaction	+	+	−
Avidity	Variable	Variable	Strong

[a] Information not available regarding possible serological activity of γD and γE.

[b] Reaction in foreign species, not in homologous species.

immunoglobulins in various reactions on the basis of information currently available. There is no intent to imply that there are no exceptions to the generalizations used in the table. As already indicated, there is conflicting evidence in some areas, and in others the data are subject to different interpretations. One can conclude, however, that γG antibodies, which are usually more apparent in later stages of immunization, are highly effective precipitins and, in most instances, account for the major portion of the complement-fixing activity of serum. They are effective in neutralizing viruses, exotoxins, and enzymes; they induce the Arthus reaction, and their ability to form a stable union with antigen increases with time after immunization.

In contrast to γG, γM antibodies, which are often the first to be detected, are most active in agglutination and lytic reactions. They are not as readily detected by precipitation and complement fixation as are γG. They also have virus-neutralizing capacity, but they apparently fail to neutralize toxins and enzymes. They seem to be less effective than γG in the Arthus reaction, and they fail to give PCA. In some instances, they have been dissociated from antigen less readily than γG, but in other reports the reverse has been noted.

The demonstrable serological activity of γA is more limited. This antibody has been demonstrated most often by direct or passive agglutination. Its ability to neutralize viruses and its relatively high concentration in secretions point to its significance in the defense mechanism.

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